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Open Science in Quantitative Bioimaging – an Oxymoron?

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Zusammenfassung

Die quantitative Bildgebung hat die Lebenswissenschaften revolutioniert, indem sie sowohl die präzise Visualisierung als auch Messung zellulärer Strukturen und Prozesse sogar bis auf molekulare Ebene ermöglicht und damit entscheidende Fortschritte in Biologie, Medizin und Wirkstoffentwicklung vorantreibt. Open Science verfolgt das Ziel, Forschung vollständig transparent und zugänglich zu machen und dadurch Reproduzierbarkeit sowie Zusammenarbeit zu fördern. Sie fordert nicht nur die frei zugängliche Veröffentlichung wissenschaftlicher Ergebnisse, sondern auch die Bereitstellung der gewonnenen Roh- und Verarbeitungsdaten sowie auch der Software und Methoden. Aktuelle Studien zeigen jedoch einen signifikanten Mangel an grundlegenden Open-Science-Kriterien hinsichtlich der Qualitätskontrolle und Reproduzierbarkeitsstandards, und zwar nicht nur in Bezug auf die quantitative Bildgebung, sondern allgemein in den Lebenswissenschaften. In diesem Beitrag wird kritisch umrissen, wie etwa Karriere- und Wettbewerbsdruck oder die Kommerzialisierung von Wissenschaft die Umsetzung von Open Science in diesem Bereich erschweren.

Schlagwörter: Quantitative Bildgebung, Datenverfügbarkeit, Reproduzierbarkeit, Qualitätskontrolle, Open Science

Abstract

Quantitative bioimaging has transformed the life sciences by enabling the precise visualization and measurement of cellular structures and processes, even at the molecular scale, driving critical advancements in biology, medicine, and drug discovery. Open science strives to make research fully transparent and accessible, promoting reproducibility and collaboration. It advocates for sharing not only results in public scientific publications but also acquired raw and processed data, software, and methodologies. Of note, recent studies reveal a concerning lack of essential open science criteria, such as fundamental quality control and reproducibility standards, not only within the field of quantitative bioimaging but generally in the life sciences. This article briefly discusses how e.g. career pressures, competition or science commercialization, challenge the realization of open science in this field.

Keywords: Quantitative Bioimaging, data availability, reproducibility, quality control, Open Science

What is Quantitative Bioimaging?

Quantitative Bioimaging (QBI) is an interdisciplinary field (biologists, physicists, engineers, mathematicians or computer scientists) focused on the measurement and analysis of biological structures using imaging-informatics techniques. Related biomedical technologies include a wide range of macroscopic, microscopic, and nanoscopic methods, from electron microscopy to optical imaging. Unlike traditional "microscopy", quantitative bioimaging focuses on extracting numerical data from images to understand cellular phenomena in a measurable, reproducible way to avoid subjectivity in image analyses.

QBI processes include multiple steps starting from sample preparation, image acquisition, image processing, and data interpretation and communication. Each of the consecutive processes are linked and carry its own sources of variability and potential bias. Consequently, QBI is not a routine work but requires ongoing experimental research, data evaluation and method refinement.

In this respect, the Optical Imaging Resource of the Institute of Molecular Biosciences Graz (IMB-Graz) is dedicated to applying and advancing optical image-informatics methodologies. The lab harbors several state-of-the-art imaging technologies, including state-of-the-art stochastic optical reconstruction (super-resolution) microscopy (STORM) or coherent anti-Stokes Raman scattering (CARS) microscopy, to enable the study and measurement of biological processes across scales, from single molecules to whole tissues and organs. Our lab develops custom imaging modalities and informatics methods for specific applications in the life sciences including fundamental methodologies (Wolinski et al. 2009; Paar et al. 2012; Pribasnig et al. 2018). In this regard, our laboratory has been performing QBI for more than fifteen years.

Role of Open Science in Quantitative Bioimaging

Error propagation is well known in science as the phenomenon whereby uncertainties in individual measurements contribute to the overall uncertainty of calculated values. Since QBI is inherently a multi-step procedure, making the accumulation of errors at various stages particularly likely. This layered complexity underlines the critical importance of rigorous documentation, machine calibration, and method validation at each step of the image-informatics process. Without such safeguards, even high-quality raw images leave unaddressed the possibility of systematic biases that erode the reliability of quantitative claims and of the scientific outcome and interpretation in general. In addition, the public availability of both raw and pro-

cessed data upon publication is particularly important. This allows other researchers to examine and compare data at different stages of the process, enabling them to identify potential differences relative to their own datasets, which can aid in interpreting any divergent results. In addition, available data serves as an experimental platform to drive progress in other disciplines.

The fundamental principle of rigorous documentation, overall correct data handling and public data availability in science is promoted by the well-known FAIR principles (Wilkinson et al. 2016), as well as by good scientific practice and research integrity policies, as outlined by various institutions, including the University of Graz and the Austrian Agency for Research Integrity ("ÖAWI") (Austrian Agency for Research Integrity – Webpages). In summary, due to the complexity of a typical image-informatics pipeline, the application of open science principles in the field of QBI is a critical factor for ensuring quality control and the correct interpretation of scientific data.

The reality: missing (meta)data, underreporting, poor reproducibility

The reality, however, often contrasts sharply with this ideal: essential (meta)data are frequently missing, methodological details are underreported, and reproducibility is poor both in QBI and the life sciences in general. In this context, Marqués et al. (2020) showed in a study that key imaging parameters (microscope settings), such as laser power, detector gain, and exposure times ("meta-data"), are routinely missing or vaguely reported making it impossible to reproduce the experiment. In this study, they defined a set of quality criteria and measured the percentage of those criteria passing in examined 240 original research articles published in eight journals. Of note, despite new reporting standards, such as "STAR Methods" and the "Nature Research Life Sciences Reporting Summary", adequate reporting of imaging details remains rare also in this high impact journals. In their analysis, only 9 % of *Developmental Cell* and 11 % of *Nature Immunology* articles provided enough information and met the set quality criteria, respectively, to attempt replication, indicating that current requirements do not sufficiently address imaging experiments. Similarly, the description of image processing routines used for quantitative analysis of datasets was found to be inadequate. Although not subject of the quality pass analysis, they noted that many articles contained little or no information about the procedures used for image processing, analysis, or quantification, basically the necessary information for interpretation of the results.

What about the availability of published raw data sets? A study by Mirko Gabelica (Gabelica et al. 2022), although not specifically focused on bioimaging, delivered sobering results. The researchers investigated how authors responded to requests for their published data. To do this, they analyzed approximately 3,500 open access articles across 333 journals, all of which required a mandatory data availability statement (DAS); 42 % of the articles included a DAS category indicating that the data sets are available on request. Of note, 93 % of the authors either did not respond or declined to share their data upon request. Similar results were obtained even when editors of a journal in the field of brain research requested raw data for a submitted manuscripts as stated by the editor-in-chief of the journal (Miyakawa 2020).

Given these limitations in bioimaging and life science research, it is perhaps not surprising that the reproducibility of life science data remains poor. Notably, Monya Baker published a survey in *Nature* in 2016 involving 1,500 scientists from various disciplines (including biology, medicine, and chemistry) regarding their ability to reproduce both their own and others' experiments. According to this survey, more than 70 % of researchers reported failing to reproduce another scientist's experiments, and over half were unable to reproduce their own results. Interestingly, although 52 % of the scientists asked agreed that there is a significant 'crisis' of reproducibility, less than 31 % thought that failure to reproduce published results means that the result is probably wrong, and that most of them still trust the published literature (Baker 2016).

These difficulties in reproducing published studies are consistent with our own experience in the bioimaging lab. When working with the same biological material and identical equipment and resources – even in the rarer cases where method description is "fully" available and with the support of experts in different fields –, we have sometimes found it challenging to reproduce published results within our specific research field.

Possible reasons for reproducibility 'crises' in Quantitative Bioimaging and beyond

In the survey of Monya Baker also for the reason for the reproducibility crisis was asked. More than 60 % said that each of two factors always or often contribute to limited reproducibility of experiments: pressure to publish and selective reporting (Baker 2016). For career advancement, (young) researchers are often expected to publish in high-impact journals (De Herde et al. 2021), which in turn prioritize novel

findings. This may lead to data dredging ("fishing" for statistical data pattern to support the own hypothesis), p-hacking (selective picking and merging data showing desired statistically relevant output) and selective reporting (ignoring findings which are against the desired hypothesis).

In QBI, data dredging can easily be performed e.g. when researchers examine images from thousands of cells to define the subcellular localization of a labeled protein. Instead of objectively analyzing all the acquired images, a user may focus only on cells that display the desired localization pattern, while ignoring or omitting cells that show a different or ambiguous distribution. When only these selected cells are imaged and discussed in the publication, while the rest are excluded, this introduces a biased, non-representative picture of the data. Such selective presentation can lead to overinterpretation of findings and undermines the reliability and reproducibility of the study.

Another important factor in this context is the protection of intellectual property and know-how for career advancement. For instance, developing a software tool for biological applications is a demanding and intellectually sophisticated task, yet it is often undervalued by both the scientific community and funding agencies, as method development alone typically receives little dedicated funding. As a result, developers may be reluctant to share fully optimized versions of their software or may withhold specific technical details necessary for optimal use, in order to maintain a competitive advantage for their own research. This tendency is not limited to software but may also be applied to cell biological methods and protocols. This behavior is also reflected by an editorial comment published in *Nature Methods* concerning the citation of software for image processing. The article emphasizes that software and algorithm development are essential to scientific progress but often remain under-credited in scientific literature (Editorial: Giving software its due 2019).

In the survey of Monya Baker, half of the researchers asked also stated insufficient method description in the lab itself and poor oversight as reasons for insufficient replication. Our lab has identified a yet underestimated factor contributing to difficulties in reproducibility: the commercialization of research infrastructures and the resulting "black box" problem. Modern optical microscopes are multi-functional machines providing different features and imaging modes. The overall operation of these devices is designed to be as user-friendly as possible, often providing default settings that are frequently accepted without modification by users lacking specific expertise. Moreover, for advanced image processing routines implemented in the system software, the underlying algorithms and their limitations are typically

unknown or poorly documented. For example, image restoration algorithms intended to compensate for unavoidable inherent artifacts of optical imaging are often accessible via a simple button press in commercial systems. However, mathematicians have shown that these algorithms can introduce significant artifacts if insufficient information is provided or if parameters are incorrectly set, i.e., image data containing distinct grades of degradation are not applicable without pre-processing. As a result, the user-friendly "black box" nature of these systems can lead users to uncritically accept artificially processed images and to misinterpretation of the acquired data. Combined with the often inadequate reporting of imaging methods (Marqués et al. 2020), limited data availability and career-related concerns (Baker 2016), these factors further contribute to the poor reproducibility frequently observed in QBI.

Fostering Open Science in the IMB-Graz Optical Imaging Lab

The adoption of open-science principles offers effective solutions to some of the challenges facing quantitative bioimaging and the broader scientific community. In our laboratory, we are actively implementing open-science practices to address issues such as incomplete method reporting, limited data availability, and barriers to the dissemination of research tools. Through these efforts, we aim to set standards for openness and integrity in optical imaging research in Graz.

Educators and principal investigators play a vital role in fostering a scientific culture aligned with open science and open source values. In my microscopy seminars, the emphasis is not merely on "pushing buttons" or operating instruments, but on understanding the inherent limitations and challenges of various optical imaging techniques. In the case of image processing, I extend this philosophy by encouraging critical thinking about every stage of the image processing workflow, and the transparency of data analysis pipelines, all within the framework of rigorous quality control. In this respect, the requirement to make all generated data openly available as an essential quality criterion for any scientific study is clearly committed to the student. For instance, simply providing overview images of a recorded cell population, a simple feature implemented in almost any modern high-end microscope systems, would significantly reduce the wide-spread data dredging problem in quantitative bioimaging. To underscore such points, relevant literature on open data practices and quality control in quantitative bio-imaging is discussed in the courses (Senft et al. 2023; Haase et al. 2022; Koho et al. 2016; Deagle et al. 2017; Jonkman et al. 2020; Rossner and Yamada 2004).

Data management is a critical factor for effective data sharing and reproducibility, particularly with respect to the availability of comprehensive metadata. To address this, we have implemented the open source data management system "OMERO" for internal use. This platform serves as a powerful tool for storing, annotating, and sharing datasets, while ensuring that detailed metadata is systematically captured and accessible (Allan et al. 2012).

For granting public access to and ensuring long-term preservation of quantitative bioimaging data, specific commercial and non-commercial repositories are available. In this context, all data from a collaborative study at our institute, which includes large amounts of imaging data, were made publicly available through Dryad, a non-profit repository (but a storage fee applies), which may also serve as a "role model" for open science practices in this field (Rauchenwald et al. 2025). However, also other repositories are available such as the BioImage Archive established by the European Bioinformatics Institute (EBI) of the European Molecular Biology Lab (EMBL) (Hartley et al. 2022). This repository is free of charge, but is mainly intended for sharing and storing ("underlying") publication-related data. In the future, the University of Graz, which is strongly committed to advancing open science, may provide secure servers in Graz in cooperation with other institutions to provide full open access to support such initiatives.

Future aspects

In Austria, Universities, and third-party funded projects are largely financed by public funds. This creates an ethical obligation to ensure that data and findings are accurate but also accessible. Furthermore, the Austrian Science Fund (FWF) mandates data management plans, and FAIR principles are increasingly tied to project funding. Institutions like the University of Graz, which supports and hosts an excellent and growing Open Science Community (https://graz-openscience.uni-graz.at/en/), are currently taking important steps to anchor openness institutionally. A study by the Institute for Advanced Studies ("IHS") in cooperation with Aarhus University on behalf of the Federal Ministry of Education, Science and Research ("BMBWF") has highlighted a troubling scepticism toward science among Austrian citizens (Starkbaum et al. 2023). Among the reasons are perceived opacity and lack of accountability. Enforcing and committing transparency and quality control in biomedical sciences including the bioimaging field is not only a scientific responsibility but a social one and may contribute to reduce the scepticism toward science in Austria.

The pressure to publish novel findings often promotes questionable research practices, including biased data analysis and selective reporting (Baker 2016), particularly evident in QBI, cannot easily be avoided as it remains a central criterion for high-impact publication. However, there is a growing number of open access and open science journals that offer an alternative, and such journals increasingly support the publication of straightforward findings that may not be novel but still contribute significantly, e.g. by excluding specific mechanisms, clarifying negative results, or refining the direction of future research. At the very least, this helps prevent valuable data from remaining unpublished and unused.

However, among all the structural and institutional hurdles discussed, it is ultimately the scientist themselves who play the most critical role. Researchers are not only data producers but also gatekeepers of transparency. Through their choice, whether to publish full protocols, provide raw and processed data, or to critically teach the limitations of their techniques, they shape the scientific culture.

Good examples can serve as motivating precedents for peers. Scientists who commit to openness not only enhance their own credibility but contribute to a broader ecosystem of trust and collaboration. This cultural shift is ethically necessary, politically relevant, and socio-politically overdue. Is Open Science in QBI now an oxymoron? No, it is not. It is merely a symptom of the current scientific system, one that urgently needs reform. The efforts of the Open Science community, including those in Graz, will help to alleviate this symptom and help to foster a more transparent and accountable scientific culture. In this respect, by joining the Coalition for Advancing Research Assessment (CoARA) (https://coara.eu/) the University of Graz underscores this commitment (https://coara.eu/coalition/membership/).

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Short biography

Heimo Wolinski is a cell biologist with expertise in molecular cell biology, high-end optical imaging, and image-informatics. As the scientific head of the Optical Imaging Lab at IMB-Graz, he leads the development and implementation of advanced imaging workflows and custom image-analysis strategies in collaboration with local and international research partners. His research focuses on organelle interactions, with particular emphasis on lipid droplet biology and their roles in cellular homeostasis and disease. His work combines genetically modified yeast models with state-of-the-art microscopy, including super-resolution imaging, and cutting-edge image-informatics approaches such as machine learning. In addition, he is actively involved in teaching molecular and cellular imaging and image processing at the University of Graz.